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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/087,466	03/01/2002	Alexander Olek	81659A	6657

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EXAMINER	
BRUSCA, JOHN S	

ART UNIT	PAPER NUMBER
1631	

MAIL DATE	DELIVERY MODE
06/05/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/087,466	OLEK ET AL.	
	Examiner	Art Unit	
	John S. Brusca	1631	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 May 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-12,14-18,20-23,25,26,30-34,36 and 41* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-12, 14-18, 20-23, 25, 26, 30-34, 36, and 41* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Status of the Claims

1. Claims 1-12, 14-18, 20-23, 25, 26, 30-34, 36, and 41 are pending.

Claims 1-12, 14-18, 20-23, 25, 26, 30-34, 36, and 41 are rejected.

Continued Examination Under 37 CFR 1.114

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 20 May 2009 has been entered.

Claim Rejections - 35 USC § 112

3. The rejection of claims 1-12, 14-18, 20-23, 25, 26, 30-34, 36, and 41 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement in the Office action mailed 17 November 2008 has been withdrawn in view of the amendment filed 20 May 2009.

4. The rejection of claims 1-12, 14-18, 20-23, 25, 26, 30-34, 36, and 41 under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement in the Office action mailed 17 November 2008 has been withdrawn in view of the amendment filed 20 May 2009.

Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

6. Claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kikyo et al. (Developmental Biology Vol. 190, pages 66-77 (1997)) as evidenced by New England Biolabs (www.neb.com/nebeconn/tech_reference/restriction_enzymes/effects_cpg_methylation (accessed 12/26/2005)) and Siegfried et al. (Current Biology, Vol. 7, pages R305-R307 (1997)) in view of Frommer et al. (Proc. Natl. Acad. Sci. USA Vol. 89, pages 1827-1831 (1992)) in view of Huang et al. (Human Molecular Genetics Vol. 8, pages 459-470 (1999)).

The claims are drawn to a method of determining differential gene expression between tissue samples and analyzing methylation states of a plurality of genes, wherein the methylation state is determined by use of a bisulfite reaction, and adding identified differentially expressed and methylated genes to a database. In some embodiments the tissue samples are biopsies of diseased patients, the samples undergo reverse transcription, the expression level of a gene in the sample was determined, differential expression was determined by representational difference analysis (e.g. by differential display), at least 100 genes are analyzed in parallel, the procedure is repeated at least 100 times, a subset of results are recorded in a database, and the methylation analysis comprises a PCR amplification.

Kikyo et al. shows in the abstract and throughout a method of analysis of mouse embryo tissue for genes that are differentially expressed between normal embryos and abnormal embryos with chromosomal translocations. A differentially expressed neuronatin (Nnat) gene was shown to be imprinted by methylation analysis. Kikyo et al. shows on pages 67-69 differential display analysis of mRNA from the embryos, and further show reverse transcriptase-PCR on pages 67-69. Ten differentially expressed bands corresponding to differentially expressed genes were observed. Two genes were identified as H19 and Nnat (see figures 1A and 1B). Kikyo et al. further noted on page 69 prior art that used subtraction hybridization to identify Nnat as a differentially expressed gene, and verified Nnat differential expression by a reverse transcriptase-polymerase chain reaction method (see figure 1C). Kikyo et al. subsequently analyzed the Nnat gene for methylation by digestion with a panel of restriction endonucleases Hind III, BssH II,

Eag I, and Sac II (see figure 6). The results presented in Kikyo et al. are equivalent to a database of results, and some figures present a subset of the results.

The New England Biolab website establishes that BssH II , Eag I and Sac II enzymes are inhibited by methylation at CpG sites.

Siegfried et al. establishes on page R305 that CpG methylation is a term of art meaning that a cytosine is methylated.

Kikyo et al. does not show use of a bisulfite reaction to determine methylation states of cytosine, or parallel analysis of up to 100 genes, or repetition of the procedure at least 100 times.

Frommer et al. states on page 1827 that cytosine methylation has long been recognized as an important factor in the silencing of genes in mammalian cells. Frommer et al. shows in the abstract and throughout a method to determine the positions of methylated cytosine residues in DNA by use of sodium bisulfite to convert cytosine to uracil in a chemical reaction (which does not react with methylated cytosine). Frommer et al. shows in page 1828 and figure 1 that their method comprises polymerase chain reactions subsequent to the sodium bisulfite treatment that produces polynucleotides suitable for sequencing reaction analysis. The sequence analysis of the amplified products reveals the presence of positions that originally contained methylated cytosine (see figures 2 and 3). Frommer et al. lists advantages of their method on page 1830, including the positive display of methylated cytosine residues, and the capacity to analyze individual strands of a DNA sample.

Huang et al. shows in the abstract that CpG methylation is known to be associated with gene silencing in cancer, and reviews the prior art showing the relationship between cancer and

methylation states of DNA on page 459. Huang et al. shows analysis of methylation states in CpG regions in cancerous versus normal controls from cell lines in figures 3, 4, 5, and 7, and primary breast tissue versus normal breast tissue in figures 6 and 8. Huang et al. shows obtaining breast tissue sample biopsies from breast tumor and normal tissue of human breast cancer patients on page 467. Huang et al. shows a database of CpG clones that exhibit altered methylation in Table 1. Huang et al. discusses the relevance of methylation state with tumorigenesis in the discussion on pages 464-467.

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the method of Kikyo et al. by use the sodium bisulfite reaction method of methylated cytosine detection of Frommer et al. because Frommer et al. shows that their method also detects methylated cytosine, and has further advantages of positive display of methylated cytosine residues and the ability to analyze individual strands of a DNA sample. It would have been further obvious to use samples of diseased tissue because Huang et al and Frommer et al. show that methylation regulates gene expression in mammalian cells and because Huang et al. shows that methylation is associated with gene silencing in cancer, and because Huang et al. shows analysis of CpG clones from normal and cancerous cell lines and primary clinical samples for differential methylation. It would have been further obvious to generate a database of any portion of the results of the method for the purpose of retaining the results for later review, as suggested by Huang et al. in table 1. It would have been further obvious to repeat the procedures as often as desired, including up to 100 times, for the purpose of analyzing additional genes and additional patient samples for differences in gene expression and

methylation. Such additional analyses would be useful for the purpose of determining the relationship between DNA methylation and breast cancer, as observed in Huang et al.

7. Claims 1, 6, 9, 16, 21, and 36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. in view of Huang et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above and further in view of Danssaert et al. (U.S. Patent No. 5,779,981).

The claims are drawn to the method of claim 1 with the further limitation that the methylation analysis comprises use of a robot or a computer device.

Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. in view of Huang et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above do not show a methylation analysis that comprises use of a robot or a computer device.

Danssaert et al. shows in column 1, lines 22-25 that polymerase chain reactions are best performed on automated devices that allow for consistent thermal cycling. Danssaert et al. shows computer controlled thermal cyclers that comprise robotic arms in column 1, line 33, column 4, and lines 39-50, column 5, lines 31-48.

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the method of Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. in view of Huang et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above by use of a computer controlled thermal cycler, optionally with robotic arms, for conducting the polymerase chain reactions

because Danssaert et al. shows that automated thermal cyclers have the advantage of providing consistent thermal cycling, and further because it is obvious to automate a manual activity (see MPEP 2144.04).

8. Claims 1 and 12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. in view of Huang et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above, and further in view of Anderson et al.

The claims are drawn to the method of claim 1 with the further limitation that both mRNA and protein levels are measured.

Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. in view of Huang et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above do not show measurement of protein levels.

Anderson et al. shows comparison of human liver gene expression by measurement of mRNA levels and corresponding protein levels (as measured by two-dimensional protein electrophoresis). Anderson et al. shows moderate levels of correlation between mRNA levels and protein levels in figures 1 and 2. Anderson et al. conclude on page 537 that determination of protein levels allows for a better understanding of multi-level gene expression control in complex organisms such as man.

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the method of Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. in view of Huang et al. as applied to claims 1-8, 10,

11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above by additional use of the protein analysis method of Anderson et al. because Anderson et al. shows that determination of correlations between mRNA and protein levels allows for better understanding of gene expression controls.

Conclusion

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to John S. Brusca whose telephone number is 571 272-0714. The examiner can normally be reached on M-F 8:30 AM - 5:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Marjorie A. Moran can be reached on 571-272-0720. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/John S. Brusca/

Primary Examiner, Art Unit 1631

jsb